I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on August 17, 2007.

Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. 1.322 AND UNDER 37 C.F.R. 1.323
Docket No. CGS-101TD1
Patent No. 7,244,568

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Andrew S. Goldsborough
Issued	:	July 17, 2007
Patent No.	:	7,244,568
For	:	Isolation of Nucleic Acid

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 C.F.R. § 1.322 (OFFICE MISTAKE)
AND UNDER 37 C.F.R. § 1.323 (APPLICANT'S MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Title page, Item (74):	Issue Fee payment sheet:
"Saliwanchik, Lloys & Saliwanchik"	--Saliwanchik, Lloyd & Saliwanchik--
Column 2, line 4:	Page 5, lines 3-4:
"(Nordhoffet al."	--(Nordhoff et al.--

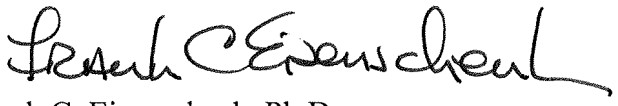
Column 3, line 9:	Page 5, line 16:
"(Potieret al."	--(Potier et al.--
Column 5, line 61:	Page 11, lines 22-23:
"2'-OII"	--2'-OH--
Column 9, lines 25-27:	Page 19, lines 8-9:
"2'-OH<formyl(—C(O)H)<acetyl(—(O)CH_3) <propanoyl (—(—C(O)CH_2CH_3)<butanoyl (—C(O)$CH_2CH_2CH_3$)"	--2'-OH < formyl (—C(O)H) < acetyl (—C(O)CH_3) < propanoyl (—C(O)CH_2CH_3) < butanoyl (—C(O)$CH_2CH_2CH_3$)--
Column 14, line 61:	Page 31, line 18:
"TIIF"	--THF--
Column 21, line 12:	Page 45, line 6:
"(10 pmol)"	--(10 μmol)--
Column 22, line 17:	Page 47, line 11:
"(65 pmol)"	--(65 μmol)--.
Patent Reads:	**Application Should Read:**
Column 27, line 63:	Preliminary Amendment dated April 8, 2004, page 3 (original claim 58), line 5:
"C_1-C_{36} C_1-C_{36} aminoalkanoyl"	--C_1-C_{36} aminoalkanoyl--.

A true and correct copy of Issue fee payment sheet and pages 5, 11, 19, 31, 45, and 47 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

The Commissioner is authorized to charge the fee of $100.00 for the amendment to Deposit Account No. 19-0065. The Commissioner is also authorized to charge any additional fees as required under 37 C.F.R. § 1.20(a) to Deposit Account No. 19-0065.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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FCE/gyl/sl

Attachments: Copy of the Issue fee payment sheet and pages 5, 11, 19, 31, 45, and 47 of the specification.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
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INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

23557 7590 03/19/2007

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Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

Sherry Loke	(Depositor's name)
Sherry Loke	(Signature)
June 13, 2007	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/820,423	04/08/2004	Andrew Simon Goldsborough	CGS-101TD1	9760

TITLE OF INVENTION: ISOLATION OF NUCLEIC ACID

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$700	$300	$0	$1000	06/19/2007

EXAMINER	ART UNIT	CLASS-SUBCLASS
TUNG, JOYCE	1637	435-006000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Saliwanchik, Lloyd & Saliwanchik
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: CYCLOPS GENOME SCIENCES LIMITED

(B) RESIDENCE: (CITY and STATE OR COUNTRY): CAMBRIDGE, UNITED KINGDOM

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☒ Issue Fee
☒ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
☐ A check is enclosed.
☒ Payment by credit card. Form PTO-2038 is attached.
☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature Frank C Eisenschenk Date June 13, 2007

Typed or printed name FRANK C. EISENSCHENK, PH.D. Registration No. 45,332

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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using mass spectrometry because a large amount of RNA degradation occurs during the analytical process. However, RNA (tRNA) up to 142 nucleotides (Nordhoff et al., (1993) Nucleic Acids Res. 21:3347; Gruic-Sovulj et al., (1997) Nucleic Acids Res. 25:1859; Tolson and Nicholson (1998) Nucleic Acids Res. 26:446) and double stranded DNA up to 500 base-pairs (Bai et al. (1995) Rapid Comm. Mass Spectrom. 9:1172; Taranenko et al., (1998) Nucleic Acids Res. 26:2488; Ausdall and Marshall (1998) Anal. Biochem. 256:220) have been measured using MALDI mass spectrometry (for reviews see; Smith (1996) Nat. Biotech. 14:1084; Murray (1996) J. of Mass Spectrom. 31:1203. Phosphate (Schuette et al., (1995) J. Pharm. Biomed. Anal. 13:1195; Sinha et al., (1994) Nucleic Acids Res. 22:3119) and chemically modified oligonucleotides (Potier et al., (1994) Nucleic Acids Res. 22:3895) have also been measured using mass spectrometry.

Although there is a molecular weight limitation to a few hundreds of nucleotides when using mass spectrometry, it provides a simple, automated means to accurately determine the exact molecular weight and therefore the percentage modification of a polynucleotide. Optimisation relies on a number of factors such as the type of mass spectrometry being carried out (electro-spray, MALDI-TOF etc), the method used to purify the modified RNA from the modification reaction, the size of the polynucleotide, the ionization matrix used, the method used to remove cations from the RNA, positive or negative ion mode and the voltage strength used (Fenn et al., 1989) Science 246:64). Capillary high performance liquid chromatography can be used prior to mass spectrometry of RNA because desalting and other purification steps are not required prior to ionization (Taniguchi and Hayashi (1998) Nucleic

thymine), to identical reaction conditions as used for RNA. It is expected that the DNA is not substantially modified as measured by incorporation of radioactivity, gel electrophoresis mobility, mass spectrometry, HPLC or any other analytical means used if the reaction is regiospecific for the 2'-OH group.

Modification at the 2'-OH position is preferably substantially regiospecific. Thus, there is preferably substantially no modification of the bases, phosphodiester bonds and/or any other position within the RNA chain. In this way, the polynucleotide retains important properties of the RNA. For example, advantageously, the polynucleotide is preferably modified so that a single strand of the polynucleotide is replicable by a nucleic acid polymerase to generate a second strand of polynucleotide complementary to the single strand.

The modification at the 2'-OH position may be such that the entire OH of the 2'C of the ribose ring is replaced by a reactant group R as in 2'-R or by OR having 2'-OR where the -O- group may or may not originate from the 2'-OH group. Accordingly, the substituent at the 2'-OH position in this case is R or OR respectively.

Various reactants or reactant combinations may be used, optionally in the presence of a catalyst, to provide these substituents, as described in further detail in the Examples below. Advantageously, the reactant comprises an acid anhydride, an acid halide, an acyl cyanide, a carboxylic acid or an N-acylimidazole, an alkoxyalkyl halide, an alkylthioalkyl halide, an alkoxyalkoxyalkyl halide, a trialkylsilane halide or a trialkylsilane

hydrophilic PVDF filters to purify RNA has already been described in U.S. Patent 5,958,677 (Sept. 99). However, this describes only the binding of RNA, which is in its unaltered form is a weakly hydrophobic molecule. Modified RNA molecules of increasing hydrophobicity can be created, as discussed above, by adding acyl groups of increasing chain length. Hydrophobicity increases in the order: 2'-OH < formyl (-C(O)H) < acetyl ($-C(O)CH_3$) < propanoyl ($-C(O)CH_2CH_3$) < butanoyl ($-C(O)CH_2CH_2CH_3$), i.e. according to the carbon chain length. Longer chain lengths than butanoyl were not tested but would be expected to follow the same trend. The increase in the strength of the hydrophobic interaction was tested by binding RNA and modified RNA to a polyvinylidene fluoride (PVDF) membrane and subjecting the bound material to increasingly stringent wash conditions (see Examples).

The reagent modifying the RNA 2'-OH group may be either free in solution or attached to a solid phase. In a particularly preferred aspect of this invention, a solid phase is employed. In this embodiment, the solid phase is not the same as the hydrophobic solid phase described above and is used as a reactant so that the solid phase itself is introduced as the substituent modifying the RNA (note the hydrophobic solid phase is employed only after the RNA is modified with a hydrophobic substituent). When using a solid phase, the modification reaction will lead to the covalent attachment of the RNA to the solid phase. This provides a convenient means to immobilize RNA to a solid phase in order to purify or analyze it. For example, RNA will be preferentially bound to the solid phase from a RNA, DNA and protein mixture such as a cell lysate or serum.

final concentration of 25ng per microlitre enhanced RNA binding, possibly by increasing the apparent concentration of the RNA. Other volume excluding compounds such as polyethylene glycol may also enhance RNA binding.

Addition of detergents and chaotropes:
In order to increase specific RNA binding whilst reducing protein binding, detergents were added to a reaction containing BCPB beads in different solvents. It was found that the addition of a final concentration of 1% SDS in DMSO or THF increased the amount of RNA bound to BCPB by 33% and 42% respectively whilst 1% SDS in DMF or toluene markedly reduced RNA binding. It was also found that except for toluene, a final concentration of 1% SDS increased the amount of ^{35}S labeled cellular protein bound to BCPB in DMF, DMSO and THF by 6.8, 2.3 and 1.85 times respectively that of a parallel reaction containing no added SDS. EDPA, pyridine or water led to low levels of RNA binding.

It was found that addition of 150mM sodium perchlorate (final concentration) to 40µl DMSO containing 3mg BCPB beads, 10µg BSA and 20ng of radiolabelled RNA increased the amount of RNA bound by 2.3 fold. Even lower sodium perchlorate concentrations of 1.5mM and 15mM (final concentration) increased RNA binding by 1.11 and 1.33 fold respectively. This effect was not caused by the high salt concentration because a parallel reaction containing 125mM NaCl instead of sodium perchlorate bound 30% less RNA.

The addition of sodium perchlorate also increases the

modification reaction:

2mg of BCPB beads were added to 40µl of THF and then 512ng of BMV RNA (Promega, US) in 2µl of water was added and briefly vortexed then incubated for 10min at 22°C. 1µl (10µmol) of acetic anhydride was then added in 20µl of THF containing 180µg of DMAP. The reaction was allowed to proceed for a further 5 min at 22°C and then stopped with 200µl of 70% ethanol and the beads collected by centrifugation at 3000rpm for 5sec and then washed a second time in 70% ethanol and twice in 200µl of water before being resuspended in 20µl of water. The secondary reactant such as acetic anhydride or acetic-formic anhydride could also carry various labels such as 14C or 3H allowing the amount of RNA to be determined using a scintillation measurement of the washed RNA-bead complex. Alternatively, the secondary reactant could be fluorescent such as isatoic or N-methylisatoic anhydrides or carry a label such as biotin permitting quantification of the amount of RNA bound to the bead. The secondary reactant would be expected to modify at least 75% or more of the 2'-OH groups so that the RNA is protected from degradation from for example ribonucleases. However, the secondary modification could also provide a means for a second purification step. For example the secondary reactant could contain a hydrophobic group or a ligand such as biotin for binding to a streptavidin bead. On release from the first solid phase such as BCPB beads the RNA would be specifically bound to a hydrophobic surface such as dodecyl-agarose beads.

Example 5

Probe hybridization to RNA immobilized on BCPB beads

reactant and a probe representing a viral sequence hybridized to the RNA-bead complex. The amount of probe hybridized to the bead would indicate the amount of viral RNA in the starting material.

Example 6

Use of bifunctional acid chlorides to immobilize RNA

To 5µl of QIAEX silica particles (Qiagen, Germany) in water was added 100µl of tetrahydrofuran (THF) and the particles washed once by centrifugation and then resuspended in 40µl of THF containing 15µl (65µmol) of sebacoyl chloride and the reaction incubated at 22°C for 3hrs. The particles were then washed twice in 100µl of THF in order to remove unreacted sebacoyl chloride and then resuspended in 20µl of THF. The sebacoyl chloride at this stage is covalently attached to the silica particles in such a way that there is one unreacted acid chloride group free to react with the RNA which is added to 20µl of the particles in 1µl of water. The reaction is allowed to proceed at 22°C for 30 minutes and then the particles washed with 200µl of 70% ethanol and twice in 200µl of water. Non-basic solvents are preferred so that the RNA is not degraded during the course of the reaction. Suitable solvents include THF, dimethyl sulphoxide and dimethyl formamide. Catalysts such as DMAP, 4-pyrrolidinopyridine, other aminopyridine catalysts, tetrabutylammonium fluoride or tributylphosphine may be used in catalytic quantities during the reaction. The covalently immobilized RNA can then be used for a variety of downstream applications such as RT-PCR amplification, hybridization and NASBA,

Example 7

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,244,568

APPLICATION NO.: 10/820,423

DATED : July 17, 2007

INVENTOR : Andrew S. Goldsborough

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Item (74):
Title page, "Saliwanchik, Lloys & Saliwanchik" should read
--Saliwanchik, Lloyd & Saliwanchik--.

Column 2,
Line 4, "(Nordhoffet al." should read --(Nordhoff et al.--.

Column 3,
Line 9, "(Potieret al." should read --(Potier et al.--.

Column 5,
Line 61, "2'-OII" should read --2'-OH--.

Column 9,
Lines 25-27, "2'-OH<formyl(—C(O)H)<acetyl(—(O)CH_3)<propanoyl (—(—C(O)CH_2CH_3)<butanoyl(—C(O)$CH_2CH_2CH_3$)" should read --2'-OH < formyl (—C(O)H) < acetyl (—C(O)CH_3) < propanoyl (—C(O)CH_2CH_3) < butanoyl (—C(O)$CH_2CH_2CH_3$)--.

Column 14,
Line 61, "TIIF" should read --THF--.

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UNITED STATES PATENT AND TRADEMARK OFFICE

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Column 27,
Line 63, "C_1-C_{36} C_1-C_{36} aminoalkanoyl" should read --C_1-C_{36} aminoalkanoyl--.

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